

In re Application of:
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Application No.: 09/454,223
Filing Date: December 09, 1999
Page 2



PATENT
Attorney Docket No. UCSD1590

AMENDMENTS TO THE CLAIMS

Please amend claims 7, 17, 18-20, 21, 22, 24, 25, and 26-28, and cancel claim 8, as set forth below.

Please withdraw claims 1-6 and 9-15, without prejudice or disclaimer.

The current listing of claims replaces all prior listings.

1. (Withdrawn) A hybrid nucleotide sequence of no more than 1528 base pairs including a sequence defining a structural gene expressing a conjoined single strand of a multimeric TNFSF-SPD fusion protein, said structural gene having a nucleotide base sequence selected from members of the group consisting of SEQ ID NO 1, SEQ ID NO 3 and SEQ ID NO 5.

2. (Withdrawn) The DNA segment according to claim 1, wherein the structural gene comprises:
a segment expressing a single hybrid amino acid chain of TNFSF-SPD, said segment having
a first SPD nucleotide base sequence of SEQ ID NO 1, from base 32 to base 799, and
a second sequence, expressing a portion of TNFSF stalk, selected from members of the group consisting of SEQ ID NO 1, from base 800 to base 1444, SEQ ID NO 3, from base 800 to base 1528, and SEQ ID NO 5, from base 800 to base 1441.

3. (Withdrawn) A recombinant DNA molecule comprising a vector operatively linked to an exogenous DNA segment defining a structural gene expressing a single amino acid chain of TNFSF-SPD, said structural gene having
a nucleotide base sequence selected from members of the group consisting of SEQ ID NO 1, SEQ ID NO 3 and SEQ ID NO 5, any functional equivalents and modification thereof,
and
an appropriate promoter for driving the expression of said structural gene in a compatible host organism.

4. (Withdrawn) The recombinant DNA molecule as described in claim 3 wherein said host organism is E. coli.

5. (Withdrawn) The recombinant DNA molecule as described in claim 3, wherein said host organism is a yeast.

6. (Withdrawn) The recombinant DNA molecule as described in claim 3, wherein said host organism is a higher plant or animal.

7. (Currently Amended) A soluble multimeric polypeptide of at least two trimer units, wherein each trimer unit comprises in reading frame a fusion protein trimer strand consisting of:

a first polypeptide comprising the first about 100 to 250 300 N-terminus residues of a collectin family scaffold protein amino acid sequence, wherein the first polypeptide comprises a hub and a body region of the collectin family scaffold protein; and

a second polypeptide comprising the last about 100 to 250 C-terminus residues of a tumor necrosis factor superfamily (TNFSF) ligand, wherein the second polypeptide comprises an extracellular domain (ECD) of the TNFSF ligand,

wherein the amino acids comprising a carbohydrate recognition domain (CRD) of the first polypeptide are replaced by the last about 100 to 250 amino acids comprising an extracellular domain of the second polypeptide

wherein the carboxy-terminal residue of the first polypeptide is operably linked to the amino-terminal residue of the second polypeptide via:

i) deletion of a carbohydrate recognition domain (CRD) of the collectin family scaffold protein and

ii) replacement of the CDR with the ECD of the TNFSF ligand,

whereby a single trimer strand spontaneously trimerizes with two additional trimer strands to form a trimer unit and the trimer unit binds at the hub to form the multimeric polypeptide.

8. (Canceled)

9. (Withdrawn) A method for preparing a CD40-SPD multimeric fusion polypeptide, comprising the steps of:

initiating a culture, in a nutrient medium, of prokaryotic or eukaryotic host cells transformed with a recombinant DNA molecule including an expression vector, appropriate for said cells, operatively linked to an exogenous DNA segment defining a structural gene for CD40-SPD ligand, said structural gene having a nucleotide base sequence of SEQ ID NO 1 from about base 32 to about base 1444; and

maintaining said culture for a time period sufficient for said cells to express said multimeric molecule.

10. (Withdrawn) A method of producing a secreted, large, biologically active, multimeric tumor necrosis factor superfamily ligand fusion protein chimera that is highly immunogenic and not readily diffusible, comprising:

introducing into a host cell a first chimeric DNA construct including a transcriptional promoter operatively linked to a first secretory signal sequence, followed downstream by, and in proper reading frame with

a first DNA sequence encoding a polypeptide chain of a first TNFSF ligand requiring multimerization for biological activity, joined to

a second DNA sequence encoding a collectin polypeptide at the site where the collectin's CRD was purposefully removed,

introducing into said host cell a second DNA construct including a transcriptional promoter operably linked to a second secretory signal sequence followed downstream by, and in proper reading frame with,

a third DNA sequence encoding a second polypeptide chain of a second TNFSF ligand, joined to

a fourth DNA sequence encoding a collectin polypeptide, wherein the collectin's CRD was purposefully removed;

growing said host cell in an appropriate growth medium under physiological conditions to allow the secretion of a large multimerized polypeptide fusion protein,

wherein said first polypeptide chain of a TNFSF-SPD protein is bound by parallel bonding of the respective collectin domain trimer to said second polypeptide chain of a different TNFSF-SPD polypeptide trimer, and wherein said multimerized polypeptide fusion protein exhibits biological activity characteristic of both membrane-attached TNFSFs; and

isolating said biologically active, multimerized TNFSF-SPD polypeptide fusion from said host cell.

11. (Withdrawn) The method according to claim 10, wherein the chimeric reactant compounds are humanized to guard against destruction by a potential human recipient's immune system.

12. (Withdrawn) A method of preparing a multimeric TNFSF-SPD ligand fusion protein, comprising:

preparing a first DNA segment coding for a strand of an exposed extracellular portion of TNFSF;

preparing a second DNA segment coding for a collectin polypeptide strand, wherein said collectin's CFD domain of the strand has been removed;

conjoining said first and second DNAs in proper reading frame, therein creating a TNFSF-collectin DNA construct;

inserting said construct into an expression vector system;

introducing said vector system into an appropriate cell in culture under suitable conditions;

harvesting and purifying spent medium from said culture; and

assaying for presence of multimeric TNFSF-collectin fusion protein.

13. (Withdrawn) A method for stimulating the immune response in potentially immunocompetent cells using multimeric TNFSF fusion proteins, comprising:

contacting said cells with said multimeric TNFSF fusion proteins, wherein said cells are induced to proliferate.

14. (Withdrawn) The method according to claim 15, wherein the cells are resting B cells.

15. (Withdrawn) A method for increasing antigenicity of cells, comprising: contacting said cells with said multimeric TNFSF fusion proteins, wherein said cells are tumor cells or HIV positive cells.

16. (Previously Presented) The multimeric polypeptide of claim 7, wherein the TNFSF ligand is selected from lymphotoxin-A (LTA), lymphotoxin-B (LTB), tumor necrosis factor (TNF), or any of TNFSF4-TNFSF18.

17. (Currently Amended) The multimeric polypeptide of claim 7, wherein the collectin family scaffold protein amino-acid sequence is selected from complement factor 1 (C1q), mannose binding protein, mannose-binding lectin type 1 (MBL1), mannose-binding lectin type 2 (MBL2), pulmonary surfactant protein A (SPA), pulmonary surfactant protein D (SPD), conglutinin, collectin 43, C-type lectin L1 (CL-L1), adipocyte complement related protein of 30 kDa (ACRP30), or hibernation specific protein 27 (Hib27).

18. (Currently Amended) The multimeric polypeptide of claim 7, wherein the trimer unit ~~is a homotrimer~~ comprises homomeric trimer strands.

19. (Currently Amended) The multimeric polypeptide of claim 7, wherein the trimer unit ~~is a heterotrimer~~ comprises heteromeric trimer strands.

20. (Currently Amended) The multimeric polypeptide of claim 7, wherein the collectin family ~~amino-acid sequence~~ scaffold protein is surfactant protein D.

21. (Previously Presented) The multimeric polypeptide of claim 7, wherein the TNFSF ligand is CD40L.

22. (Currently Amended) The multimeric polypeptide of claim 7, wherein the trimer unit ~~unit~~ strand is SPD-CD40L.

23. (Previously Presented) The multimeric polypeptide of claim 7, wherein the TNFSF ligand is receptor activator of NF-kappaB ligand (RANKL).

24. (Currently Amended) The multimeric polypeptide of claim 7, wherein the trimer ~~unit~~ strand is SPD-RANKL.

25. (Previously Presented) The multimeric polypeptide of claim 7, wherein the TNFSF ligand is CD27L/CD70L.

26. (Currently Amended) The multimeric polypeptide of claim 7, wherein the trimer ~~unit~~ strand is SPD-CD27L/CD70L.

27. (Currently Amended) The multimeric polypeptide of claim 7, wherein amino acid residues comprising the trimerized strands which are susceptible to sites of proteolytic degradation are removed from the multimeric polypeptide.

28. (Currently Amended) The multimeric polypeptide of claim 7, wherein the multimer is a dimer of trimer units.